



Phytochemical Profiling Using GCMS And Antioxidant Activity of *Alpinia Purpurata* Flower Extract

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Abstract:

Chronic diseases are the major cause of death worldwide and its incidence increases day by day due to lifestyle as well as environmental factors. The present study carried out the screening of bioactive compounds present in *Alpinia purpurata* flower extract by using Gas Chromatography - Mass Spectroscopy (GC-MS). Plants with their medicinal properties play an important role in food and pharmaceutical industry, one such asset of nature is *Alpinia purpurata* an ancient ayurvedic herb belonging to *Zingiberacia* family that is known for its cardioprotective due to presence of various polyphenolic compounds such as alkaloids, flavonoids, phenolics, tannins and glycosides. The present study mainly focused on the screening of bioactive compounds present in *Alpinia purpurata* flower extract using GC-MS investigation. Twenty four compounds were identified and they were capable of various pharmacological activities like anti-inflammatory, anti-cancer, anti-arthritic etc. This study will provide future directions in the field of development of food products using *Alpinia purpurata* flower extract.

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Keywords: -*Alpinia purpurata*. Antioxidant activity, GC-MS, *Zingiberacia*, pharmacological activities.

INTRODUCTION

The plants still show a beneficial role in preventing various human pathological. Isolation and extraction of plant compounds are vitally important to understand their impact on the prevention and treatment of various illnesses [1]. Many reports have been executed on natural antioxidants for decades to provide protection against diseases linked to oxidative stress and to the various damages induced by the presence of free radicals. Generally, oxidative stress is associated with several diseases such as cancer, neurodegenerative diseases, diabetes and inflammatory diseases, and ageing process [2].

Actually, most of the plants showed a various range of compounds with antioxidant activities such as polyphenols as secondary metabolites. This component has been shown as strongly natural antioxidants of the medicinal world [3]. Currently, they are used against heart conditions, headaches, colds, wounds, and various skin infections, as well as insecticides and herbicides.

One of the largest families in the plant kingdom is Zingiberaceae. It is the major natural resource which provides many useful products for food, spices, medicines, dyes, perfume and aesthetics to man. *Alpinia* has more than 200 species and is the largest genus of the family [4]. *Alpinia purpurata* (Vieill.) K. Schum (red

ginger) is a herbaceous plant, internationally seen in the ornamental plant market as potted plant, landscape accent and cut flower. Ginger is mainly a creeping perennial on a thick tuberous rhizome. In the first year, a green, erect, reed-like stem about 60 cm high grows from this rhizome [5].



Fig: 1. *Alpinia purpurata*

Zingiberaceae species contain a wide range of essential oils [6]. *Alpinia* species are well known medicinal herbs that have been proven by previous researches to have several effects, namely anti-inflammatory [7], antioxidant, antimicrobial, antidermatophytic [8], antinociceptive [9], hepatoprotective [10], immunostimulatory [11] and anticancer activities [12]. *Alpinia* is the principal genus in the ginger family in which *Alpinia purpurata* (Vieill.) K. Schum is a very popular garden plant in India [13]. The present study carried out the screening of the bioactive compounds present in *Alpinia purpurata* flower extract using GC-MS. In our study will provide future directions in the field of development of food products using *Alpinia purpurata* flower extract. *Alpinia purpurata*, better known for its ornamental use, has been assessed for medicinal potential, and various parts of the plant have bioactive compounds with therapeutic efficacy and most of the bioactive compounds can act as antioxidants, anticancer agents, anti-inflammatory agents, and neuroprotective agents [14].

MATERIALS AND METHODS

Preparation of different flower extracts

The *Alpinia purpurata* flower was collected from Peelamedu Coimbatore district, TamilNadu, India. It was authenticated from the botanical survey of India, Coimbatore. NO. BSI/SRC/5/23//2023/Tech – 556

Method of Extraction

Extraction is the crucial first step in the analysis of medicinal plants, because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. 20% of the extract was prepared in dissolving the different solvent of methanol, ethanol, and water (20g in 100ml). After dissolving, the flask was incubated in an orbital shaker for 24 hrs with 60 to 70 rpm at 40°C. After incubation the extract was filtered and used for further study.

Phytochemical Analysis

Alkaloids (Mayer's test)

To 1 ml of extract was added to 1 ml of Mayer reagent (0.113g of mercuric chloride added in 5ml of D.H₂O). A few drops of iodine were added, development of yellow colour indicates the presence of alkaloids.

Terpenoids (H₂SO₄ test)

To the 1 ml of extract add with 1 ml of concentrated H₂SO₄, keep the tube in a water bath for 2 to 4 minutes forming a greyish colour indicating the presence of terpenoids.

Phenol (FeCl₂ test)

1 ml of extract was added with 1ml of FeCl₃, formation of blue green or black that indicates the presence of tannin.

Glucose

1 ml of extract were mixed with 1ml of Fehling's solution A (0.35g of CuSO₄ in 5ml of D.H₂O) and 1 ml of Fehling's solution B (1.75 potassium sodium tartrate and 0.5g of NaOH in 5 ml of D.H₂O), kept in water bath for 2 to 4 minutes. Formation of Red colour indicates the presence of sugar.

Saponins (Foam test)

1 ml of extract was added with 1 or 2 ml of D.H₂O. Shake vigorously and the formation of a 1cm foam layer indicates the presence of saponins.

Flavonoids

To the 1 ml of extract a few fragments of magnesium ribbon and few drops of concentrated HCl were added. A pink scarlet colour was appearing to confirm the presence of flavonoids.

Quinines

Take 1ml of extract, with 1ml of 2% of NaOH were added. Formation of blue green colour, it indicates the presence of quinines.

Protein (Folin's lowry's method)

Take 1ml of extract and add few drops of mercuric chloride or nitric acid, formation of yellow colour, indicates the presence of phenol.[15,16]

Steroids H₂SO₄ test

To 1 ml of extract, add 1 ml of chloroform and concH₂SO₄ in sidewise, a red colour present at the lower chloroform layer that indicates the presence of steroids.

Quantitative analysis and antioxidant contents**Flavonoids**

Each 1 ml of extract was pipetted out and added with 0.1ml of 10% AlCl₃ and 0.1ml of 1M potassium sodium tartrate, and added 2.8 ml of D.H₂O, then it was incubated at room temperature for 30 minutes. The absorbance was read at 415 nm by using spectrophotometer Quercetin was used as standard to calculate the mg/g of the flavonoid content. [17]

Total phenol test

The Folin and Ciocalteu method were used to determine the total phenolic contents of the extract. First, 500µl of the sample was dissolved in 0.5ml of the folin-ciocalteu reagent over 3 minutes. Then, 2 ml of 20% (w/v) sodium carbonate was added to the mixture. The mixture was left to stand in the dark place for 60 minutes and absorbance at 650 nm. Calibration curves were used to calculate the total phenolic concentration using gallic acid equivalent / g dry weight.[18]

DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity

Various concentrations of *Alpinia purpurata* of the sample (4.0 ml) were mixed with 1.0 ml of methanol solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM . The mixture was shaken vigorously and left to stand for 30 minutes and the absorbance was measured at 517 nm. AA was used as a control. The percentage of DPPH decolorization of the sample was calculated according to the equation

$$\text{DPPH radical scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{test}}) / A_{\text{control}}] \times 100$$

IC₅₀ values (mg extract/ml) were the inhibitory concentration at which DPPH radicals were scavenged by 50%. AA was used for comparison.[19].

Ferric-reducing antioxidant power assay (FRAP)

A stock solution of 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40 mM HCL, 20mM FeCl₃.6H₂O and 0.3M acetate buffer (pH 3.6) was prepared [20]. The FRAP reagent contained 2.5 ml TPTZ solution, 2.5 ml ferric chloride solution, and 25 ml acetate buffer. It was freshly prepared and warmed to 37°C. FRAP reagent (900 ml) was mixed with 90 ml water and 30 ml *Alpinia purpurata* of the sample and standard antioxidant solution. The reaction mixture was then incubated at 37 °C for 30 minutes and the absorbance was recorded at 595 nm. An intense blue color complex was formed when ferric tripyridyltriazine (Fe³⁺-TPTZ) complex was reduced to ferrous (Fe²⁺) form. The absorption at 540 nm was recorded.

Superoxide anion radical scavenging activity

This assay was based on the reduction of nitro blue tetrazolium (NBT) in the presence of nicotinamide adenine dinucleotide (NADH) and phenazine methosulfate (PMS) under aerobic conditions [21]. The 3 ml reaction mixture contained 50 ml of 1M NBT, 150 ml of 1M NADH with or without sample, and Tris buffer (0.02M, pH 8.0). The reaction was started by adding 15 ml of 1M PMS to the mixture and the absorbance change was recorded at 560 nm after 2 minutes. Percent inhibition was calculated against a control without the extract

Reducing power assay

The reducing power was determined as described by Yen and Chen. Briefly, 0.13 ml of *Alpinia purpurata* different concentration (5-50 µg/ml) in phosphate buffer (0.2 M, pH 6.6) were mixed with 0.125 ml of potassium ferricyanide (1%, w/v) and incubated at 50°C for 20 min. Afterwards, 0.125 ml of TCA (10%, w/v) was added to the mixture to terminate the reaction. Then, the solution was mixed with 1.5 ml ferric chloride (0.1%, w/v) and the absorbance was measured at 700 nm.

Purification and Characterization of the extract

Fourier Transform Infrared Red (FTIR)

FTIR was used to identify the characteristic functional groups in the extract. It provides the information about the structure of a molecule that could frequently be obtained from its absorption spectrum. A small quantity of the sample was placed and scanned. The IR spectrum was obtained using the Shimadzu infrared spectrometer, it was scanned from 400 to 4000 cm.

Thin -Layer Chromatography

The extracts were added as spot using capillary tubes on the one end of the TLC plate (gel 60 F₂₅₄ 20 X 20 cm) at above 1 cm. Plate was allowed it for air dry, then it was placed in a beaker containing a solvent of Acetonitrile: water: formic acid in the ratio of 0.7:1.3:0.1. The samples were allowed to run towards the other end of the plate. The sheet was removed and allowed it to air dry. Calculated R_f value using the given below;[22]

$$R_f \text{ value} = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$$

GC MS Analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising an AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument. Total GC running time is 51.25min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0. Interpretation on GCMS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST and WILEY 7 library. The name, molecular weight and structure of the components of the test materials were ascertained [23].

RESULTS AND DISCUSSION

Qualitative analysis of *Alpinia purpurata* flower extract

To study the phytochemical composition of the extracts prepared from *Alpinia purpurata*, a phytochemical screening was performed allowing to consider the possible medical uses that may have this plant as several studies have demonstrated the positive correlation between the phytochemical composition of plants and their medicinal uses [24]. Qualitative preliminary phytochemical analysis the results confirm the presence of alkaloids, saponin, flavonoids, steroids, terpenoids, phenols, quinines, proteins in the hydro-ethanolic and aqueous extract. The methanolic extracts showed the presence of steroids, terpenoids, phenols, saponins, flavonoids, quinines. Among the various extracts, the hydro-ethanolic extract contains higher concentration of phytochemical constituents (table1).

Table : 1 Phytochemical screening of *Alpinia purpurata* flower extract

Test name	<i>Alpinia purpurata</i> flower extract		
	Aqueous	Methanol	Ethanol
Alkaloids	++	++	++
Terpenoids	+	+	+
Phenol	+++	+++	+++
Sugar	-	-	-
Saponins	+++	+	+
Flavonoids	+++	+++	+++
Quinines	+	+	+
Proteins	++	-	++
Steroids	++	-	++

(+)- Present (-) Absent

Consequently, *Alpinia purpurata* by its richness in different secondary metabolites may have several medical importances such as anti-tumor, especially the ethanol extract due to the presence of flavonoids [25], antioxidants due to its richness in phenolic compounds [26]. The secondary metabolites were quantified, and the total phenol, total tannin, total flavonoid, *Alpinia purpurata*, methanol, for good antioxidant activity (Table 2).

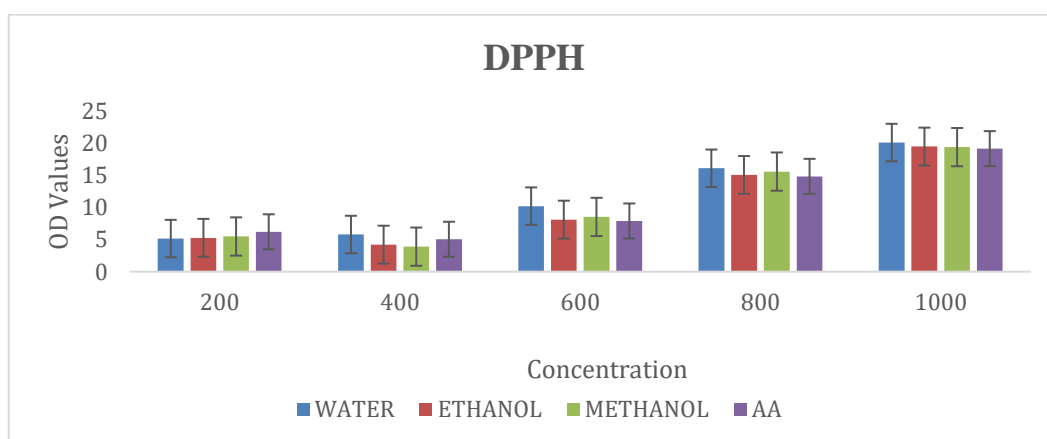
Table : 2 Quantitative analysis and antioxidant contents Flavonoids and Phenolic

Sample	Total Flavonoid in (mg)	Total Phenolic content in (mg)
Water	155	146
Methanol	170	150
Ethanol	162	75

DPPH (2,2-Diphenyl-1-picrylhydrazyl)

DPPH have been used extensively as a free radical to evaluate reducing substances [27]. This purple color generally fades/disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them to a colorless/bleached product (i.e., DPPH or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm. Hence, the more rapidly the absorbance decreases, the more potent antioxidant activity of the extract.

The DPPH radical assay is a suitable model for estimating radical scavenging activities of antioxidants. *Alpinia purpurata* exhibited a significant dose dependent inhibition of DPPH activity. This had a lesser activity than the standard of AA. The results are presented in Fig.2 the IC₅₀ value of AA and *Alpinia purpurata* was 18.42 µg/ml, 20.56 µg/ml, respectively.

**Fig. 2: DPPH radical scavenging ability of *Alpinia purpurata***

FRAP

Ferric-reducing power is an important indicator of the antioxidant potential of a compound or an extract [29]. The ability to reduce ferric ions indicates that the antioxidant compounds are electron donors and could reduce

the oxidized intermediate of lipid peroxidation processes, thus acting as primary and secondary antioxidants [30,31]. The antioxidant activity of the methanolic crude extract determined by FRAP assay varied as seen in Fig.3. The reducing power was found to be higher in **Aqueous** extract. At a concentration of 37.23 $\mu\text{g/ml}$ of *Alpinia purpurata* 50% of FRAP generated by incubation was scavenged. The IC_{50} value of AA was 35.58 $\mu\text{g/ml}$.

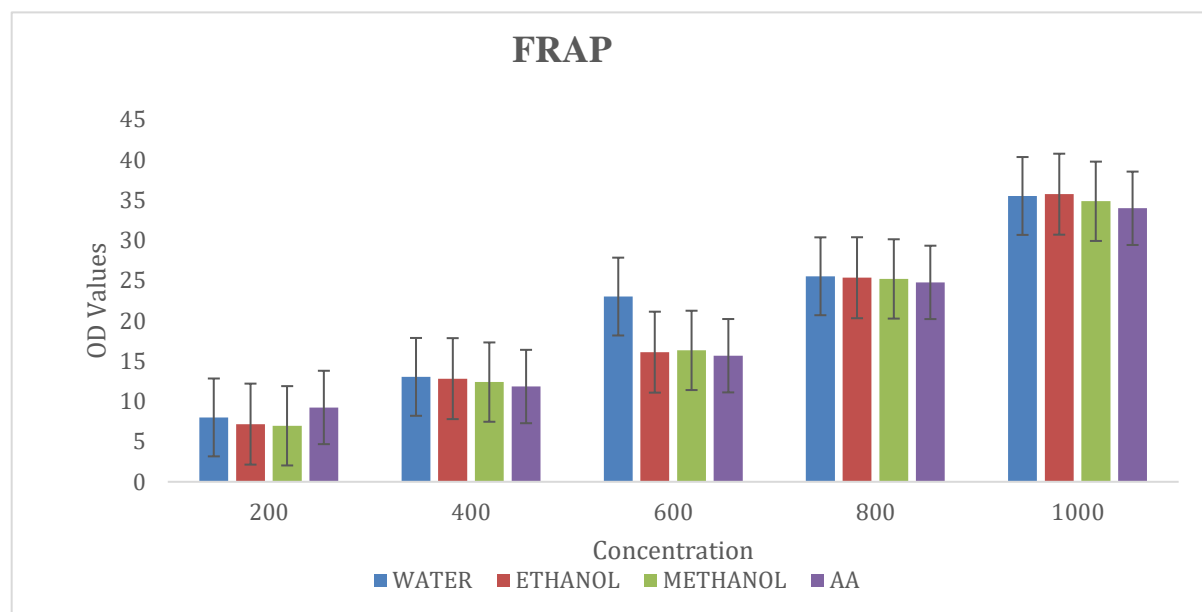


Fig. 3: FRAP radical scavenging ability of *Alpinia purpurata*

SUPEROXIDE ANION RADICAL SCAVENGING ASSAY:

Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals that are generated after the oxygen is taken into living cells. Superoxide anion changes to other harmful ROS and free radicals such as hydrogen peroxide and hydroxyl radical, which induce oxidative damage [31]. The decrease the absorbance at 560 nm with the *Alpinia purpurata* thus indicates the consumption of superoxide anion in the reaction mixture. The antioxidant activity of the methanol crude extract determined by Superoxide anion radical assay varied as seen in Fig.4. The reducing power was found to be higher in **Aqueous** extract. At a concentration of 38.05 $\mu\text{g/ml}$ of *Alpinia purpurata* 50% of Superoxide anion radical generated by incubation was scavenged. The IC_{50} value of AA was 35.58 $\mu\text{g/ml}$, respectively.

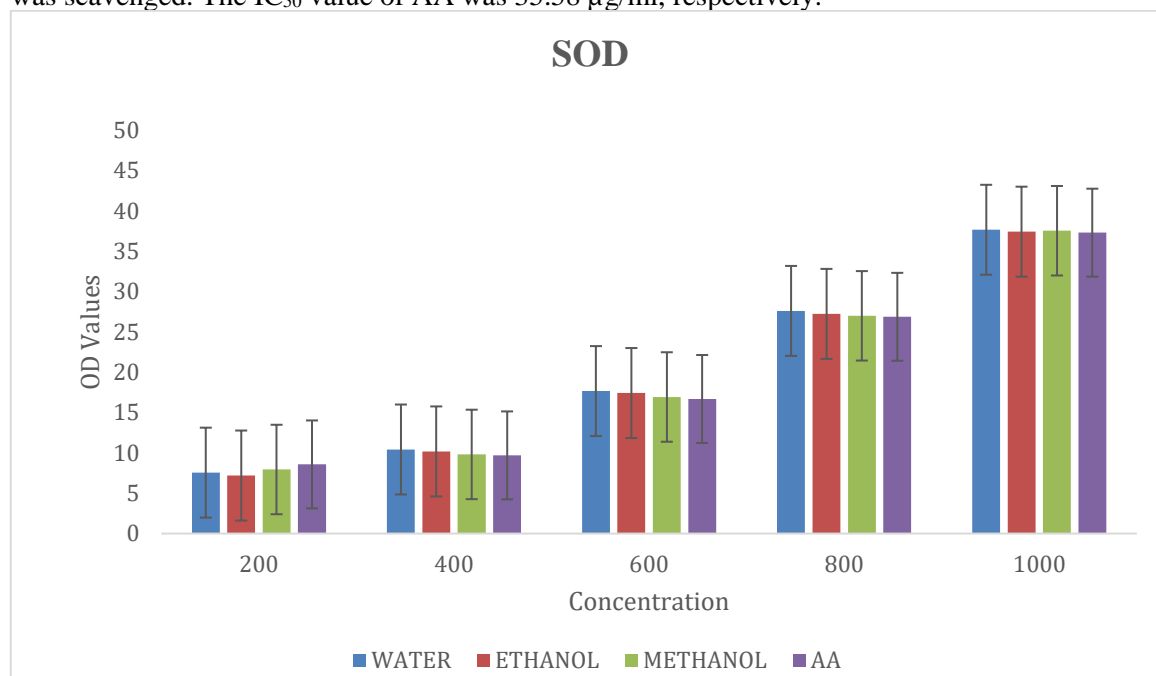


Fig. 4: Superoxide anion radical scavenging ability of *Alpinia purpurata*

REDUCING POWER ASSAY

Reducing capacity is considered as a significant indicator of potential antioxidant activity of a compound or sample [32]. The presence of reductants (i.e. Antioxidants) causes the reduction of the Fe^{3+} /ferricyanide complex to the ferrous form. Therefore, by measuring the formation of Per's Prussian blue at 655 nm, the amount of Fe^{2+} can be monitored. Higher absorbance indicated higher reducing power [33]. Fig.5 elucidated the reduced capabilities of *Alpinia purpurata* compared to AA. The reducing power of *Alpinia purpurata* was increased in quantity of sample. *Alpinia purpurata* could reduce the most Fe^{3+} ions, which had a lesser reductive activity than the standard of AA. The IC_{50} value of *Alpinia purpurata* and AA was 32.58 $\mu\text{g/ml}$ and 35.28 $\mu\text{g/ml}$ respectively.

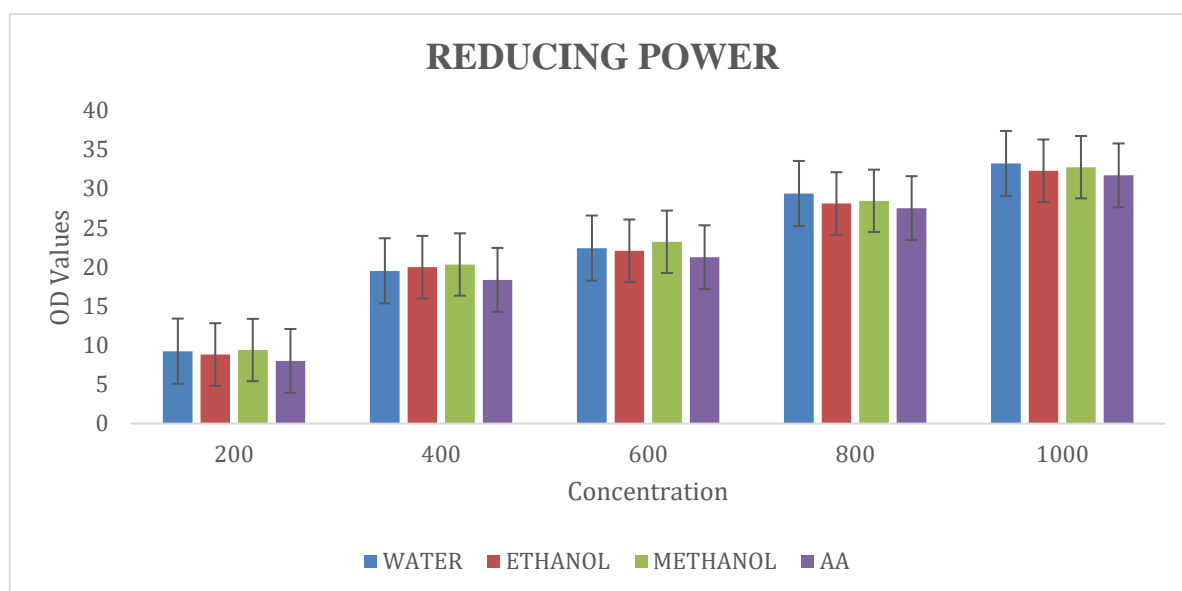


Fig. 5: Reducing power radical scavenging ability of *Alpinia purpurata*

FT-IR

FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The Infra-red spectroscopic (IR) analysis of study *Alpinia purpurata*, in a bandwidth ranging from 400 to 4000 cm^{-1} , revealed the presence of different functional groups (Figure 6,7,8). The peaks showed that the flower extract of *Alpinia purpurata* may have the compounds like OH group, Alkanes, C-H group, C-H stretching, Carbonyl group, CH bending, Aliphatic amines, Carboxylic acid compounds (Table 3,4,5). The above result was analyzed with interpretation of infrared spectra, a practical approach (34,35,36). This analytical method is rapid, highly effective, visual and accurate for pharmaceutical research. Analysis of the flower extract of *Alpinia purpurata* sample under FT-IR technique showed that the presence of phenolic compound and flavonoid which can be isolated and further screened for different kind of biological activities depending their therapeutic uses. Further research will be needed to find out the structural analysis of flavonoid compound by the use of different analytical methods such as NMR, HPLC, UPLC.

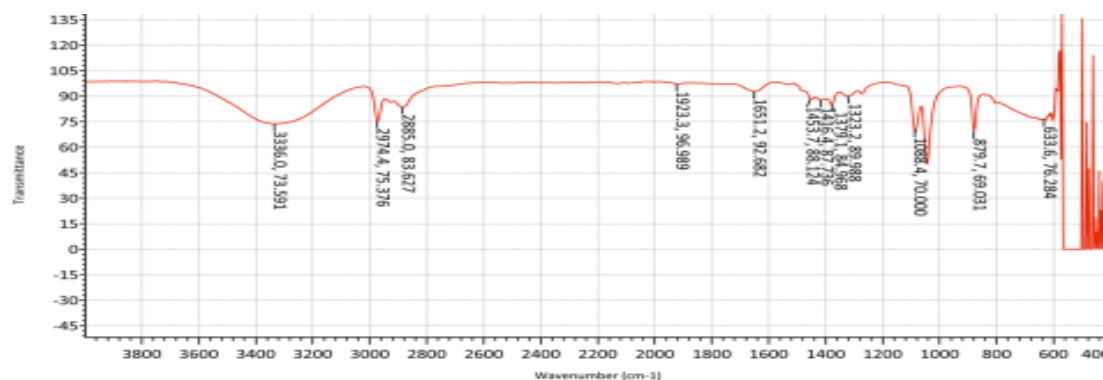
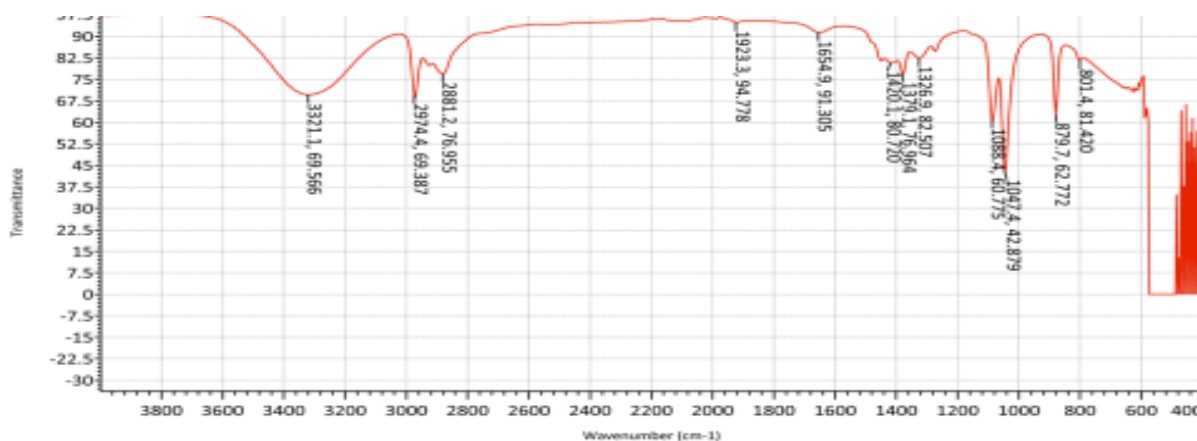


Fig. 6: FTIR Ethanol of *Alpinia purpurata* flower extracts

Table. 3: FTIR Ethanol peak value of *Alpinia purpurata* flower extracts

Peak Number	Wavenumber (cm ⁻¹)	Intensity	Functional Group
1	633.6	76.284	C-Cl (stretching, halo compound)
2	879.7	69.031	C-Cl (stretching, halo compound)
3	1088.4	70.000	C-F (stretching, fluoro compound)
4	1323.2	89.988	O-H (bending, phenol)
5	1379.1	84.968	O-H (bending, phenol)
6	1416.4	87.736	O-H (bending, alcohol)
7	1453.7	88.124	O-H (bending, carboxylic acid)
8	1651.2	92.682	C=C (stretching, cyclic alkene)
9	1923.3	96.989	C=C=C (stretching, allene)
10	2885.0	83.627	C-H (stretching, alkane)
11	2974.4	75.376	C-H (stretching, alkane)
12	3336.0	73.591	N-H(stretching, aliphatic primary amine)

**Fig. 7: FTIR methanol of *Alpinia purpurata* flower extracts****Table. 4: FTIR methanol peak value of *Alpinia purpurata* flower extracts**

Peak Number	Wavenumber (cm ⁻¹)	Intensity	Functional group
1	801.4	81.420	C-Cl(stretching, halo compound)
2	879.7	62.772	C-Cl(stretching, halo compound)
3	1047.4	42.879	S=O (stretching, sulfoxide)
4	1088.4	60.775	C-O (stretching, secondary alcohol)
5	1326.9	82.507	S=O (stretching, sulfone)

6	1379.1	76.964	O-H (bending, phenol)
7	1420.1	80.720	O-H (bending, alcohol)
8	1654.9	91.305	C=C (stretching, alkene)
9	1923.3	94.778	C=C=C (stretching, allene)
10	2881.2	76.955	C-H (stretching, alkane)
11	2974.4	69.387	O-H (stretching, alcohol)
12	3321.1	69.566	O-H (stretching, alcohol)

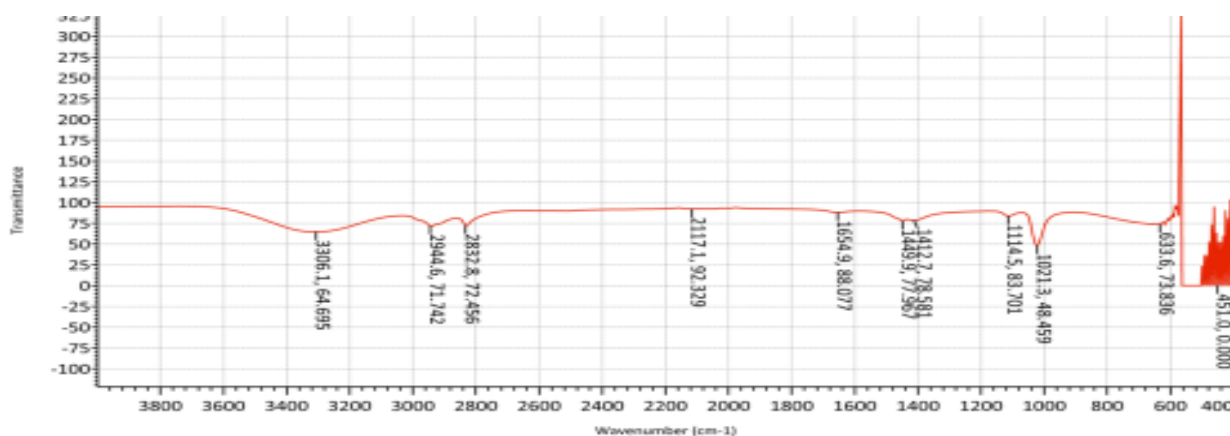


Fig. 8: FTIR water of *Alpinia purpurata* flower extracts

Table. 5: FTIR water peak value of *Alpinia purpurata* flower extracts

Peak Number	Wavenumber (cm ⁻¹)	Intensity	Functional group
1	451.0	0.000	C-I
2	633.6	73.836	C-Br stretching, halo compound
3	1021.3	48.459	C=C bending, alene
4	1114.5	83.701	C=C bending, alene
5	1412.7	78.581	S=O stretching, sulfite
6	1449.9	77.967	C-H bending, alkane
7	1654.9	88.077	C=C stretching, alkene
8	2117.1	92.329	N=C=S stretching, isothiocyanate
9	2832.8	72.456	S-H stretching, thiol
10	2944.6	71.742	C-H stretching, alkane
11	3306.1	64.695	N-H stretching, aliphatic primary amine

TLC



Methanol
Spot 1 -Rf value - 0.607
Spot 2-Rf value - 0.823



Ethanol
Spot 1 -Rf value - 0.821



Water
Spot 1 -Rf value -0.535
Spot 2-Rf value - 0.821

GC MS Analysis of *Alpinia purpurata* flower extract

Twenty three compounds were identified in hydro-ethanolic extract by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in Table 6. The prevailing compounds are (2-Aminophenyl)(pyrrolidin-1-yl) methanone, O-Desmethyl-cis-tramadol, 2-[p-Aminophenyl] benzimidazole, Pentanoic acid, 2-(E)-Hexenoic acid, Pyrolo [3,2 d]pyrimidin-2,4(1H,3H)-dione, Oxime-, methoxy-phenyl-, 2-Cyclopenten-1-one, 2-hydroxy-, Cyclotetrasiloxane, octamethyl-, D-Limonene, p-Cyanophenylp-(2-methylbutoxy) benzoate, 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, 2-Pentenoic acid, 4-oxo-, methyl ester, (Z)-, Squalene, Octadecanoic acid, Phthalic acid, butyl 2-pentyl ester, Benzamide, 3,4-difluoro-N-(3,4-difluorobenzoyl)-N-ethyl-. However, isolation of individual phytochemical constituents and subjecting its biological activity

Table .6: GC MS Analysis of *Alpinia purpurata* flower extract

S.No.	R. Time	Compound Name	Formula	Component Area	Match Factor
1	3.1774	(2- Aminophenyl)(pyrrolidin-1-yl)methanone	C ₁₁ H ₁₄ N ₂ O	211519.1	50.9
2	3.2248	O-Desmethyl-cis-tramadol	C ₁₅ H ₂₃ NO ₂	694614.3	51.1
3	3.4880	2-[p-Aminophenyl]benzimidazole	C ₁₃ H ₁₁ N ₃	146664.0	55.2
4	4.5882	Pentanoic Acid	C ₅ H ₁₀ O ₂	27030.1	52.1
5	5.1159	Oxime-,methoxy-phenyl-	C ₈ H ₉ NO ₂	4156180.4	76.4
6	5.5564	2-Cyclopenten-1-one,2-hydroxy-,Cyclotetrasiloxane	C ₅ H ₆ O ₂	861090.2	71.2

7	7.6799	octamethyl-,D-Limonene	C ₁₀ H ₁₆	911820.5	80.2
8	32.4622	9,12-Octadecadienoic acid (Z,Z)-	C ₂₁ H ₃₈ O ₄	3305060.9	60.9
9	25.3966	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	18233798.1	93.9
10	4.5882	2-Pentenoic acid	C ₅ H ₁₀ O ₂	27030.1	52.1
11	27.6351	Squalene	C ₃₀ H ₅₀	998652.2	57.6
12	27.4467	9-Octadecenoic acid,	C ₁₈ H ₃₄ O ₂	6613698.5	83.9
13	31.3389	Phthalic acid	C ₂₄ H ₃₈ O ₄	1980185.5	87.6

CONCLUSION

The present work was focused on the bioactive compounds present in *Alpinia purpurata* flower extract and its antioxidant activity. *Alpinia purpurata* flower extracts contained high amounts of phenols, flavonoids, tannins and anthocyanins, also, exhibited the strong antioxidant potential. In this study, *Alpinia purpurata* flower extracts is a valuable source of health-promoting compounds, fulfilling concurrently the promising antioxidant activity that can be utilized virtually as food complements, to tardiness lipid oxidation and healing from particular ailments via its free-radicals scavenging ability.

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